

Supplementary Materials

Tables S1-S4

Figures S1-S3

Table S1. *B. hermsii* infection of mice immunized with recombinant BHA007 or FlaB^a

Antigen	Mouse	Days spirochetes seen in blood ^b	Antibodies to FhaB by dot blot	Mouse weight (gm)	Spleen (% body wt)	Mean 16S rDNA copies/µg in spleen DNA (95% CI) ^c	Mean 16S rDNA copies/µg in liver DNA (95% CI)
BHA007	1	None	-	21.5	1.58	- ^d	8 (2-31)
BHA007	2	5	+	21.3	1.65	1510 (499-4560)	47200 (15700-142000)
BHA007	3	4,5	+	21.1	1.33	3980 (1340-11800)	801 (261-2460)
BHA007	4	4,5,7	+	22	1.73	927 (213-4040)	75 (22-253)
BHA007	5	None	-	21.6	0.46	-	-
BHA007	6	None	-	23.5	0.47	-	-
FlaB	7	None	-	21.4	0.42	-	-
FlaB	8	None	+	23	2.00	368 (83-1640)	313 (99-992)
FlaB	9	4,5,7,8	+	21.5	1.63	12 (2-59)	14 (4-52)
FlaB	10	8	+	21.2	1.32	341 (76-1520)	178 (55-578)

^a Unless noted, all of the measurements as well as sera tissues taken for further analysis was performed on day 18 of infection.^b days 3, 4, 5, 7, and 8 of tail vein blood was examined by phase-contrast microscopy.^c CI, confidence interval.^d -, mean 16S rDNA copies/µg < 5.

Table S2. Percent amino acid sequence identity of BHA007 of *B. hermsii* and orthologous proteins of other *Borrelia* species

	<i>hermsii</i>	<i>parkeri</i>	<i>turicatae</i>	<i>duttonii</i>	<i>recurrentis</i>	<i>crocidurae</i>
<i>hermsii</i>	-	60	56	44	44	42
<i>parkeri</i>		-	79	49	48	45
<i>turicatae</i>			-	46	46	45
<i>duttonii</i>				-	91	91
<i>recurrentis</i>					-	91
<i>crocidurae</i>						-

Table S3. Selected characteristics of processed BHA007 and orthologous proteins of other *Borrelia* species

	<i>hermsii</i>	<i>parkeri</i>	<i>turicatae</i>	<i>duttonii</i>	<i>recurrentis</i>	<i>crocidurae</i>
Residues	356	342	350	338	338	339
Asp + Glu	50	50	56	62	63	61
Arg + Lys	52	48	43	48	47	47
Daltons	38,755	38,021	38,938	38,151	38,280	38,383
Isoelectric Point	8.3	5.8	4.9	4.9	4.8	4.9

Table S4. Percentages of predicted secondary structure of processed BHA007 of *B. hermsii* and orthologous proteins of other *Borrelia* species

	<i>hermsii</i>	<i>parkeri</i>	<i>turicatae</i>	<i>duttonii</i>	<i>recurrentis</i>	<i>crocidurae</i>
Alpha helix	47	38	37	46	50	47
Beta strand	13	7	5	13	10	10
Random coil	40	56	58	41	40	43

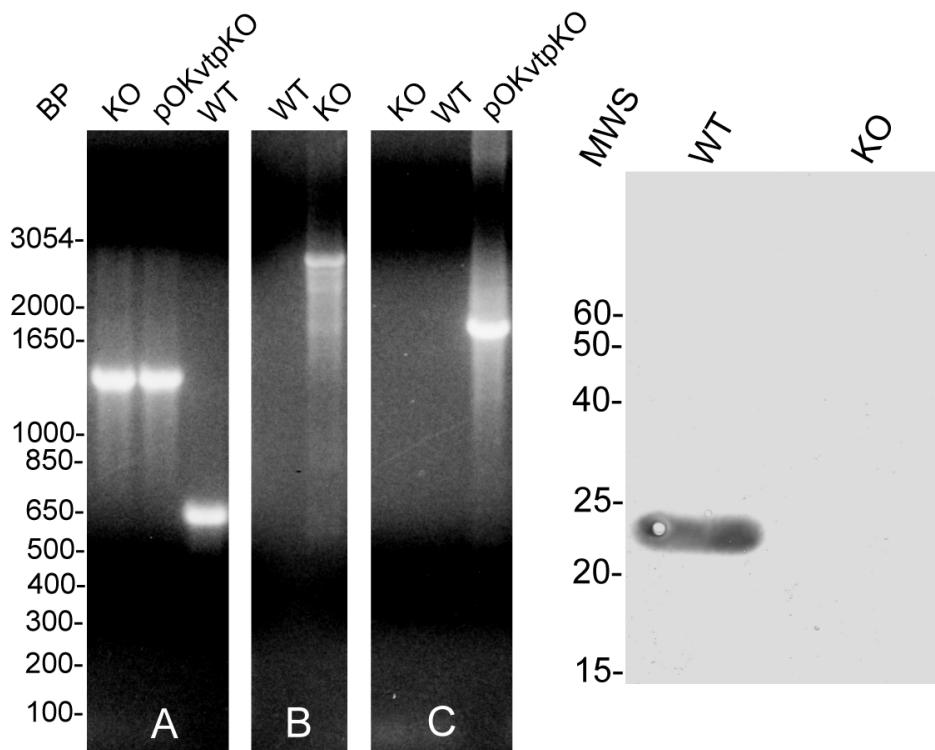


Figure S1. PCR analysis and Western blot assay provide evidence of the insertion of the *flgB*-kanamycin cassette into the *vtp* gene of *B. hermsii*.

Left panel: PCR with primers (see Methods) for full-length *vtp* gene of putative Vtp-knock-out (KO), the plasmid for creating the knock-out (pOKvtpKO), and Vtp+ wild-type (WT) cells (agarose gel A); PCR with primers within the kanamycin cassette and within the *B. hermsii* plasmid outside the *vtp* gene (gel B); and PCR with primers for sequence within the plasmid vector and not in either the *flgB*-kanamycin cassette or *vtp* gene (gel C). The locations of size standards in base pairs (BP) are shown on the left.

Right panel: Western blot analysis of WT and KO cells with a Vtp-specific monoclonal antibody. The locations of the molecular weight standards (MWS) in kilodaltons are shown on the left.

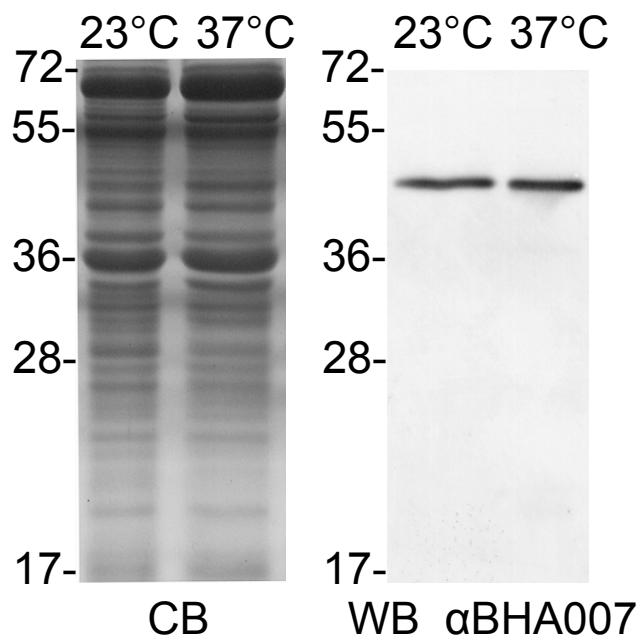


Figure S2. Expression of BHA007 in cells of Vtp- knock-out at 23°C and 37°C. The left panel is a Coomassie Blue-stained PAGE of cell lysates of the Vtp- knock-out cultivated at 23°C or 37°C. The right panel is a Western blot (WB) of duplicate lanes of the gel with rabbit antiserum to rBHA007. Detection of bound antibodies was with alkaline phosphatase-labeled Protein A/G. The locations of the molecular weight standards (MWS) are shown on the left of each panel.

Bb_BBK32	(FbpA)	YYLDEY-----DEEDE EEIRLSNRYQSYLEGVKYNVDSAQTI T ^{KIYNTY} TL
Bt_BBK32	(FbpA)	YYLEDDDEDEY-----DE EEIRLDNRYS TYKSYLESVRYNVGSAINTIDKIFRNYIL
Bd_BBK32	(FbpA)	YYWEDNEDSWEDK-----ELL KEEIRLDNRYS TYKSYLESVRYNVDSAIDTIDK ^{IYNNY} IL
Bh_BBK321k	(FbpB)	EE-DDDNYGEYE-----DEDDK EEARLDKEY KSRSRKTNSVKALEIARQIRYDWGT
Bt_BBK321k	(FbpB)	MTIEEDEFEEGE-----DTNEKE EETRLDNQY KFKLNKTDSVKKALKIAGKIKDDWAK
Bh_BHA007	(FbpC)	- SAIISGFSGSM ----- TEEEE <u>DPRYEY</u> YDQLKAEKD IDSAFKILEKLKKDRDQ
Bt_BTA001	(FbpC)	IPTTISGSASSI -----IE EEDSPQYEY YDQLEEAETVDSALKLIQKIKGDRDQ
Bd_CihC	(FbpC)	SPTVSGSYSGS-----IVE EEDSPEYEY NDKLD ^{SAEKD} IDYVLEVIEQIKKDRNQ

Figure S3. Alignment of partial protein sequences of BBK32, BBK32-like (lk), and BHA007-like proteins (BTA001 and CihC) of *Borrelia burgdorferi* (Bb), *B. turicatae* (Bt), *B. duttonii* (Bd), or *B. hermsii* (Bh). Terms in parentheses are proposed individual names for this family of proteins that are homologous to BBK32. Accession numbers for *B. burgdorferi*'s BBK32 protein and *B. duttonii*'s CihC protein were AE000788 and FN552440, respectively. Other accession numbers are given in Methods. A conserved motif in all sequences is indicated by red text. The residues in blue text were identified as part of the fibronectin binding domain by reference 60 (Brenner C, Bomans K, Habicht J, Simon MM, and Wallich R. 2013. Mapping the ligand-binding region of *Borrelia hermsii* fibronectin-binding protein. *PLoS One* 8:e63437).